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Technical report

# The Vitek analyser for routine bacterial identification and susceptibility testing: protocols, problems, and pitfalls

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**Abstract**

**Automated and semiautomated technology in microbiology has seen great advances in recent years. The choice of automated equipment for the identification and susceptibility testing of bacteria in a routine diagnostic laboratory depends on speed, accuracy, ease of use, and cost factors. The Vitek analyser (bioMerieux, UK) was installed in a busy diagnostic teaching hospital laboratory in London. This report describes one year's experience. Changes to work practice as a result of incorporating the equipment into the laboratory, and the advantages and disadvantages of automation in key areas are described in detail, together with possible solutions to problems. The Vitek analyser was found to be valuable for the speed and accuracy with which results were available for the common bacterial pathogens. Results of susceptibility testing were standardised according to NCCLS guidelines and used breakpoint MICs to ascertain susceptibility and resistance; they were an improvement on disc testing. This equipment is not a reference facility for difficult to identify organisms and many manual techniques, including some disc susceptibility testing, will have to be retained by the laboratory.**

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Keywords: Vitek analyser; automation; bacterial identification and susceptibility testing

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Rapid and reliable identification of microorganisms is essential for the effective management of infectious diseases. Early diagnosis and treatment is crucial, reducing both morbidity and mortality and the danger of spread of infection. In a cost conscious environment, specific, targeted management and early discharge from hospital is highly desirable. During the last decade many automated and semiautomated bacterial identification and susceptibility systems have been launched. The choice of one system over another depends on the speed with which accurate identification and susceptibility results are made available to the clinician, ease of use, spectrum of organisms covered, maintenance and quality control costs, and the presence of a versatile software package. This report describes our experiences with the Vitek system (bioMerieux, UK) in the one year since its inception into a busy microbiology diagnostic laboratory in the United Kingdom. It is worth noting that the choice and range of antibiotics on susceptibility testing cards were custom made for the University College London Hospitals (UCLH).

## Identification and susceptibility testing on the Vitek system

Identification of microorganisms is accomplished by biochemical methods. A turbidometrically controlled suspension of pure colonies in saline is inoculated into identification cards. These cards contain 29 different biochemical broths in reaction cells and one negative control cell to assess growth and viability of the suspension. Conventional catalase, coagulase, and oxidase tests (where appropriate) and the results of a Gram stain are required before inoculation of cards. Incubation times vary from two to 15 hours depending on the growth rate of the organism. The Vitek programmed computer determines whether each well is positive or negative by measuring light attenuation with an optical scanner. When the incubation period is completed, the reactions are analysed automatically and the identification is printed.

Antimicrobial susceptibility tests are run similarly on cards which contain dilutions of antimicrobials to determine the breakpoint minimum inhibitory concentration (MIC) against the organisms. There are separate cards for Gram positive and Gram negative organisms. The MIC cutoff values differentiating sensitive, moderate, and resistant status for an organism against appropriate antimicrobials are programmed into the system and are based on the NCCLS, USA guidelines.[1] A comparison of these values with those published by the BSAC[2] yielded many differences. A decision was made use the NCCLS guidelines because it was felt that these values reflected a stringently controlled, standardised, and widely accepted set of guidelines.

*Table 1 Vitek card use (February 1996 to January 1997)*

| *Card description* | *Total use (mainly on urine bench)* | *Projected use (all specimen types)* | *Modifications in card type and/or use* |
|---|---|---|---|
| *Gram negative organisms* | | | |
| • Identification (GNI) | 4600 | 6840 | No change in card number or type ordered for the next year |
| • Susceptibility | | | |
| Routine-GNS-PW* | 400 | 3220 | Change in card type (new GNS-QL card) |
| Urine-GNS-PX* | 4340 | 5000 | No changes |
| *Gram positive organisms* | | | |
| • Identification (GPI) | 600 | 7720 | No changes |
| • Susceptibility Streptococci (GPS-AH)* | 400 | 1140 | Terminated further orders |
| Staphylococci (GPS-AI)* | 400 | 7460 | Changed to incorporate staphylococci and streptococci (new GPS-AM card)* |

*See table 2.

## Initiation of the Vitek system into the laboratory and analysis of card use

The Vitek system was initially used exclusively for the identification and susceptibility testing of Gram negative isolates from urine specimens. In the period ranging from February 1996 to January 1997 the laboratory received over 41 000 urine culture requests. The actual and projected analysis of card usage in the laboratory is illustrated in table 1. A review of the system at the end of one year showed the need for changes in card type. In our experience such a review and the flexibility to make major antimicrobial changes (table 2) was crucial to the continued use of the system in the laboratory.

One of the undisputed advantages of the Vitek, if loaded with cards early in the day, is the speed with which it yields an identification and susceptibility test result on a Gram negative isolate, sometimes in less than four hours. The system also obviates the need to read API strips and disc diffusion plates the next day, thus saving several hours.

An ideal hospital computer system would be able to incorporate the Vitek laboratory computer interfaced with the hospital mainframe, so that results when validated are automatically downloaded and available to user screens throughout the hospital, saving on the considerable time spent in entering results. A new pathology computer system for the whole Trust is imminent at the UCLH and will be automatically interfaced with equipment such as the Vitek analyser. At present, results are entered manually. Accurate assessments of turnaround times and savings related to labour can only be made when the interface is finally in place.

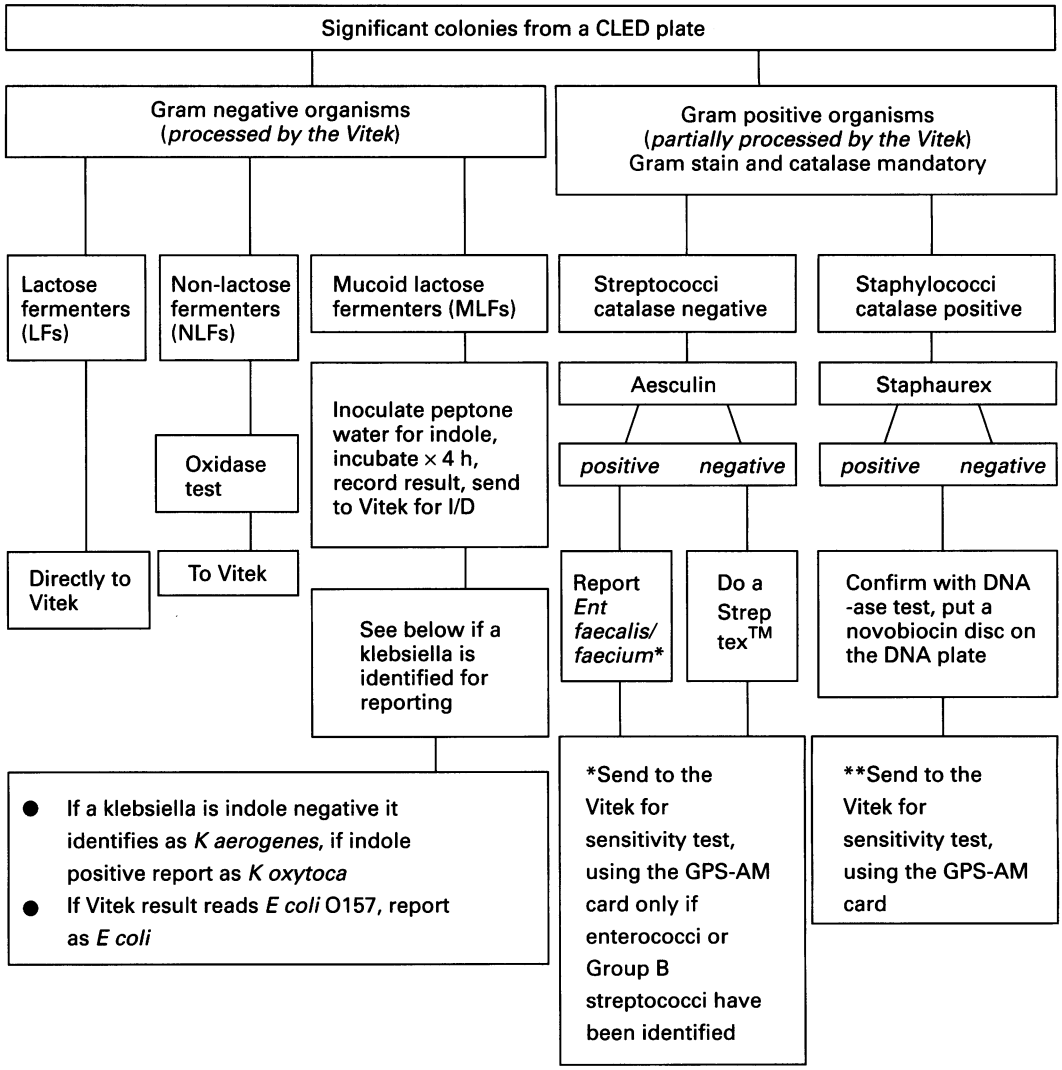
## Identification protocols using the Vitek system

To minimise duplication of work and maximise efficiency it was decided that each bench would be provided with flow diagrams for quick and easy reference. The following flow diagrams illustrate the changes in microbiology practice that were a direct result of the acquisition of the Vitek system (figs 1–6).

There were a few minor problems. Possible *E coli* 0157 was being identified in urine more often than with conventional methods (because sorbitol is incorporated in the panel of biochemical tests used for identification). Serological confirmation of these isolates

*Table 2 Custom made susceptibility test cards for UCLH use*

| *GNS-PW Routine coliforms from sources other than urine and all pseudomonads* | *GNS-PX Gram negative isolates in the urine other than pseudomonads* | *GPS -AI All staphylococcal isolates except those from urine* | *GPS-AH streptococcal isolates: only group B and enterococci from sources other than urine* |
|---|---|---|---|
| Ampicillin | Ampicillin | Penicillin | Penicillin |
| Azlocillin | Co-amoxiclav | Oxacillin | Ampicillin |
| Piperacillin | Cephalothin | Erythromycin | Erythromycin |
| Imipenem | Cefuroxime | Fusidic acid | Chloramphenicol |
| Cefuroxime | Nalidixic acid | Tetracycline | Tetracycline |
| Ceftazidime | Ciprofloxacin | Trimethoprim | Rifampicin |
| Ciprofloxacin | Gentamicin | Gentamicin | Gentamicin 500 |
| Gentamicin | Nitrofurantoin | Amikacin | Streptomycin 2000 (equivalent to high level gentamicin) |
| Amikacin | Tetracycline | Teicoplanin | Teicoplanin |
| Trimethoprim | Trimethoprim | Vancomycin | Vancomycin |
| | Extended spectrum B-lactamases (ESBL) | B lactamase | |
| *Changes in card types following Vitek audit of 1996–1997* | | | |
| **New GNS-QL card** | No changes | **New GPS-AM card for all relevant gram positive isolates from urine and other specimens** | Further orders terminated |
| Ampicillin | | Penicillin | |
| Co-amoxiclav | | Oxacillin | |
| Piperacillin | | Erythromycin | |
| Piptazobactam | | Gentamicin | |
| Imipenem | | Fusidic acid | |
| Cefuroxime | | Teicoplanin | |
| Ceftazidime | | Vancomycin | |
| Ciprofloxacin | | Trimethoprim | |
| Gentamicin | | Nitrofurantoin | |
| ESBL | | Tetracycline | |
| | | β-Lactamase | |




*Group B streptococci: record the following susceptibilities: penicillin (report amoxycillin), nitrofurantoin, tetracycline, trimethoprim (disc test result). Cephaloridine, cefuroxime ciprofloxacin will be put by disc only when requested.

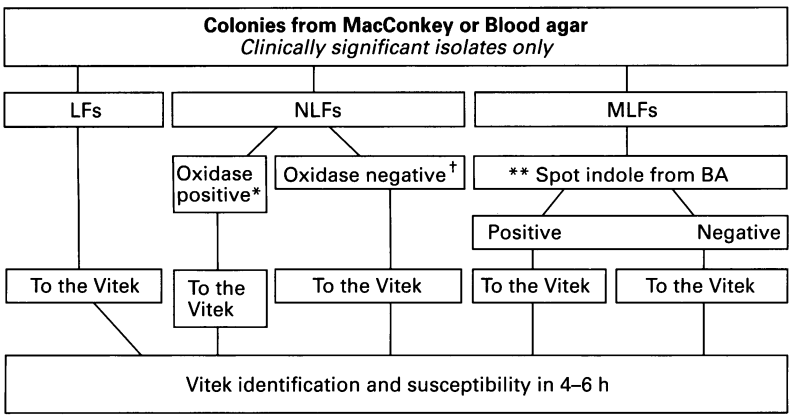
Aesculin positive streptococci: penicillin (report amoxicillin), nitrofurantoin, tetracycline, vancomycin, teicoplanin, trimethoprim (disc test result). Gentamicin 10 and 100 need to be put up by disc only when requested. If amoxycillin sensitive, report as *Ent faecalis*, if moderate or resistant send it to the Vitek to confirm *Ent faecium* identity

** For all staphylococci record the following susceptibilities: penicillin (report amoxicillin) oxacillin (report flucloxacillin), gentamicin, nitrofurantoin, trimethoprim, tetracycline. Cefuroxime, cephaloridine, ciprofloxacin will be put up by disc only when requested.

If DNAase negative, irrespective of Staphaurex™ result, check novobiocin. If resistant report *Staph saprophyticus*, if sensitive (and DNAase and Staphaurex™ negative) report coagulase negative staphylococci.

*Flowchart 1 Processing urine specimens through the Vitek.*

with O157 antisera was always negative, so it was decided that further investigation by serology would only be done on isolates from stool samples. *Acinetobacter* spp were not identified reliably; this occurred primarily with the inactive strains, where the Vitek result yielded either an unidentified organism or an organism with low discrimination. Identification in such cases was on based on the oxidative, alkaline, and inactive changes seen in Hugh-Leifson's oxidative/fermentative medium.




* Process direct through Vitek if growth is pure/heavy. If not, confirm significance with medical staff and put up purity plate. If necessary a pseudomonas selective agar (PSM) can be used.
** The indole positive differentiates *K oxytoca* from other klebsiellae.
† If the Vitek identification reads *Stenotrophomonas maltophilia* a manual sensitivity needs to be put up as the Vitek does not have a reliable susceptibility test for this organism. Acinetobacter identification can also be unreliable, when no identification is possible put a Hugh-Leifson test.

Azlocillin susceptibility is only available for pseudomonads, all other gram negative isolates needing azlocillin (as specified by medical staff) should be tested by a manual disc diffusion test.

*Flowchart 2 Identification of gram negative isolates from all sources other than urine. LFs, lactose fermenters; NLFs, non-lactose fermenters; MLF, mucoid lactose fermenter; BA, blood agar.*

*Staphylococcus saprophyticus* from urine was not reliably identified; all coagulase (Staphaurex™) positive, DNAase negative strains of staphylococci were identifying as *S aureus*. The streptococci yielded a sparse and sometimes mixed growth of urethral flora on urine cysteine-lysine-electrolyte deficient (CLED) plates and it was more convenient and cost–effective to do an aesculin test to make a presumptive identification (flowchart 1).

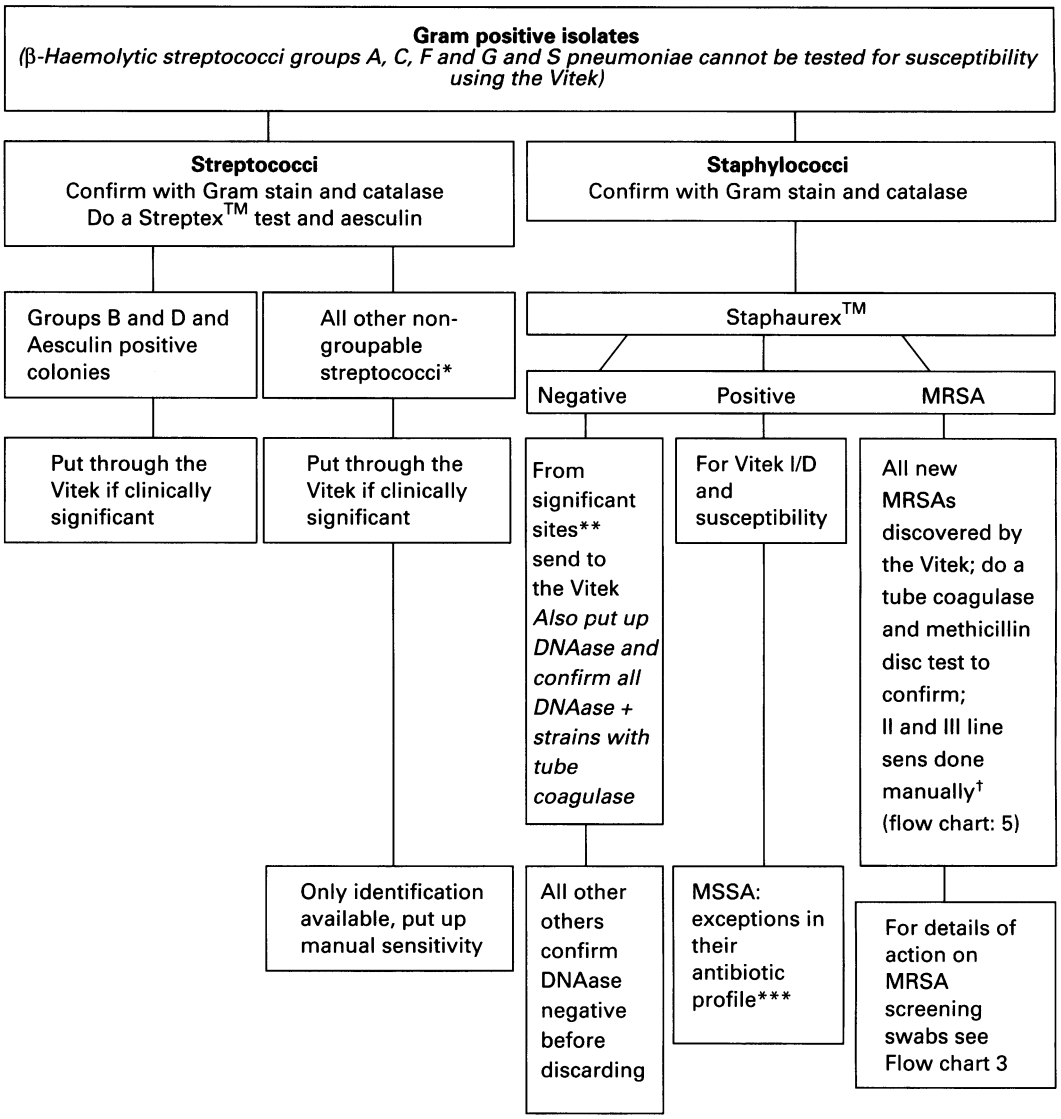
Even though streptococci belonging to Lancefield groups A, C, F, and G, *Str pneumoniae*, and the "viridans" group of streptococci are identified by the Vitek, susceptibility testing is not yet available on this system. It was found to be more cost–effective and less labour intensive to use conventional methods of testing for all of the above organisms except the "viridans" group of streptococci, which were speciated by the Vitek when clinically relevant. The identification of *S aureus* (from all sources) on the Vitek depended on the result of the Staphaurex™ test done manually before loading the identification card. To preclude the possibility of laboratory error or the occurrence of rare false positives or false negatives, supplementary tests were included in the testing protocol, as depicted in flowchart 3. Full identification of *S aureus* was possible within six hours and of methicillin resistant *S aureus* (MRSA) from a mannitol-salt-agar plate within nine hours.

Blood cultures are currently processed using the Bactec 9240 system (Becton Dickinson, Oxford, UK). When bottles signal positive, the broth culture is subjected to conventional testing (Option 2 in flowchart 4). In our experience the Vitek does not offer any advantage over API (appareil procédes d'identification) testing, especially for Gram negative isolates from blood cultures (option 1 in flowchart 4). The Vitek was used only for the identification of coagulase negative staphylococci, the enterococci, and the "viridans" group streptococci from a purity plate.

*Table 3 Antibiotic testing policy in the UCLH as done by disc diffusion*

| *Staphylococci* | | *Streptococci including enterococci and pneumococci* | *Gram negative rods: blood and wounds* | |
|---|---|---|---|---|
| *First line* | *Second line* | | *First line* | *Second line* |
| Penicillin | Amikacin | Oxacillin (only pneumococci) | Amoxycillin | Co-amoxiclav |
| Erythromycin | Ciprofloxacin | Amoxycillin | Trimethoprim | Amikacin* |
| Methicillin | Ceftazidime | †Gentamicin 10 and 100 | Gentamicin* | Azlocillin* |
| Teicoplanin | Vancomycin | Sulphonamide | Ciprofloxacin* | Meropenem* |
| Gentamicin | Piperacillin | Trimethoprim | Cefuroxime | Piperacillin* |
| Fusidic acid | Piptazobactam (this line has now been abandoned) | Teicoplanin<br>Second line as for staphylococci<br>†*only for enterococci* | Ceftazidime*<br>**used for pseudomonas* | Piptazobactam* |
| *MRSA* | *Faeces* | *Group A streptococci* | *CSF* | *Urine* |
| Penicillin | Amoxycillin | Penicillin | Penicillin | Amoxycillin |
| Erythromycin | Ciprofloxacin | Erythromycin | Chloramphenicol | Sulphonamide |
| Methicillin | Trimethoprim | Tetracycline | Cefotaxime | Trimethoprim |
| Gentamicin | Nalidixic acid | | Amoxycillin | Gentamicin |
| Fusidin | Chloramphenicol | | Rifampicin | Cefuroxime |
| Clindamycin | | | Sulphonamide | Nalidixic acid |
| Tobramycin | | | Ciprofloxacin | Nitrofurantoin |
| Trimethoprim | | | | Cephaloridine |
| Mupirocin | | | | Tetracycline |
| Rifampicin | | | | Augmentin |
| Ciprofloxacin | | | | Ciprofloxacin** |
| Amikacin | | | | |
| Tetracycline | | | | |
| Vancomycin | | | | |
| Novobiocin | | | | |
| Kanamycin | | | | |

**For staphylococci in urine add penicillin, methicillin, novobiocin.
For faecal streptococci in urine, omit cephaloridine and add gentamicin 100 μg, vancomycin 5 μg and 30 μg, and teicoplanin.




* This includes the Viridans group and other streptococci not readily identified by the Streptex ™ test.

** Staphaurex ™ negative staphylococci from significant sites: Line tips, prostheses, endocarditis, Continuous ambulatory peritoneal dialysis (CAPD) specimens, sternal wounds, and other clinically relevant specimens as indicated by medical staff.

*** Exceptions to the antibiotic testing profile for methicillin sensitive *Staph aureus* (MSSA):

In the eye: chloramphenicol and neomycin are put up manually on all specimens at the same time as inoculating the Vitek tube. For other sites chloramphenicol is tested manually only if requested by medical staff.

† For known MRSAs second and third line sensitivities are only done if they have not been done in the preceding month.

*Flowchart 3 Identification of gram positive isolates from all sources other than urine. I/D, identification; MRSA, methicillin resistant S aureus.*

## Susceptibility testing

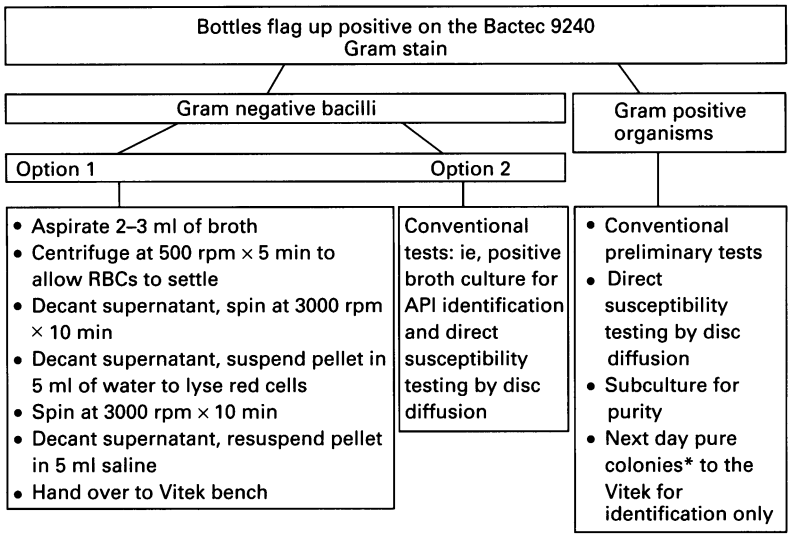
The first change requested for antibiotic cards customised for UCLH use was the replacement of cotrimoxazole (trimethoprim + sulphamethoxazole) by trimethoprim alone. Following this, other susceptibility cards were designed keeping in mind our current antibiotic policy. Table 2 lists the antibiotics specially compiled for the UCLH diagnostic laboratory in 1996 and the changes incorporated in 1997 after one year's experience with these cards. Table 3 lists the range of antibiotics previously in use in the laboratory, when testing was done by the Stokes comparative method.[3]

### REPORT ON THE USE OF CUSTOM MADE CARDS

One year into the use of custom made cards, we audited the successes, problems, and pitfalls of this system, bench by bench.

*Urine bench*

The use of the GNS-PX card (table 2) was very successful, in that identification and

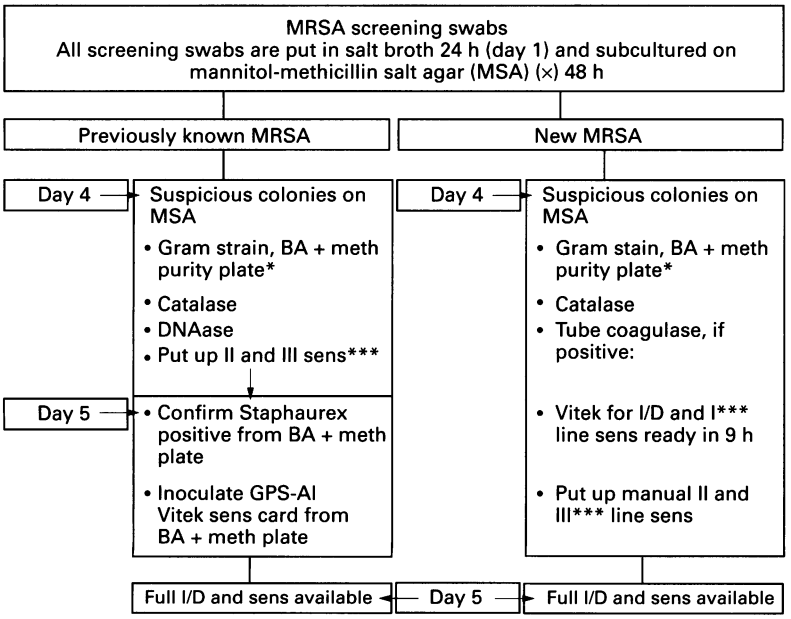



* Only significant isolates of coagulase negative staphylococci and the "viridans" group streptococci need be identified by the Vitek. *Staph aureus* continues to be identified and susceptibility tested by conventional methods.

Note: Option 1 is laborious, takes 45 minutes in preparation. If done early in the day, results will be available the same day. If positives flag up later in the day, no time is gained as compared to option 2. It also needs the use of a sealed centrifuge in the blood culture room.

*Flowchart 4 Processing blood cultures using the Vitek system. API, appareil procédes d'identification; RBCs, red blood cells.*

susceptibility was available within four to six hours of obtaining a significant isolate in pure culture. For an oxidase positive isolate, the GNS-PW card was used and the non-pseudomonal antibiotics were ignored (table 2). The Vitek system has recently introduced a "catch all" Gram positive sensitivity card, the GPS-AM card (table 2). Depending on the source and final identification of the isolate, only the appropriate antibiotic susceptibility




* Blood agar with methicillin disc.
** II and III sensitivities are only needed if they have not done in the preceding month.

*** I line susceptibility: Vitek GPS-AM card.
II line: clindamycin, netilmicin, sulphonamide, chloramphenicol, tobramycin.
III line: mupirocin, ciprofloxacin, rifampicin, novobiocin, kanamycin,

*Flowchart 5 Methicillin resistant S aureus (MRSA) screening swabs. BA, blood agar; meth, methicillin.*

test results required for treatment are reported, as in flowchart 1. Any extra antibiotics needed would have to be done by the disc method and only if peculiar to the patient's needs. At present the GPS-AM card can be used for all staphylococci, group B streptococci, and enterococci. A manual trimethoprim disc susceptibility test needs to be done on all streptococci in the urine, as the Vitek does not release trimethoprim test results on these organisms. A gentamicin test result on the Vitek, even when available, is not validated for streptococci and should not be used. Low and high level gentamicin resistances in enterococci need to be determined by supplementary disc tests when indicated.

*Wound bench*

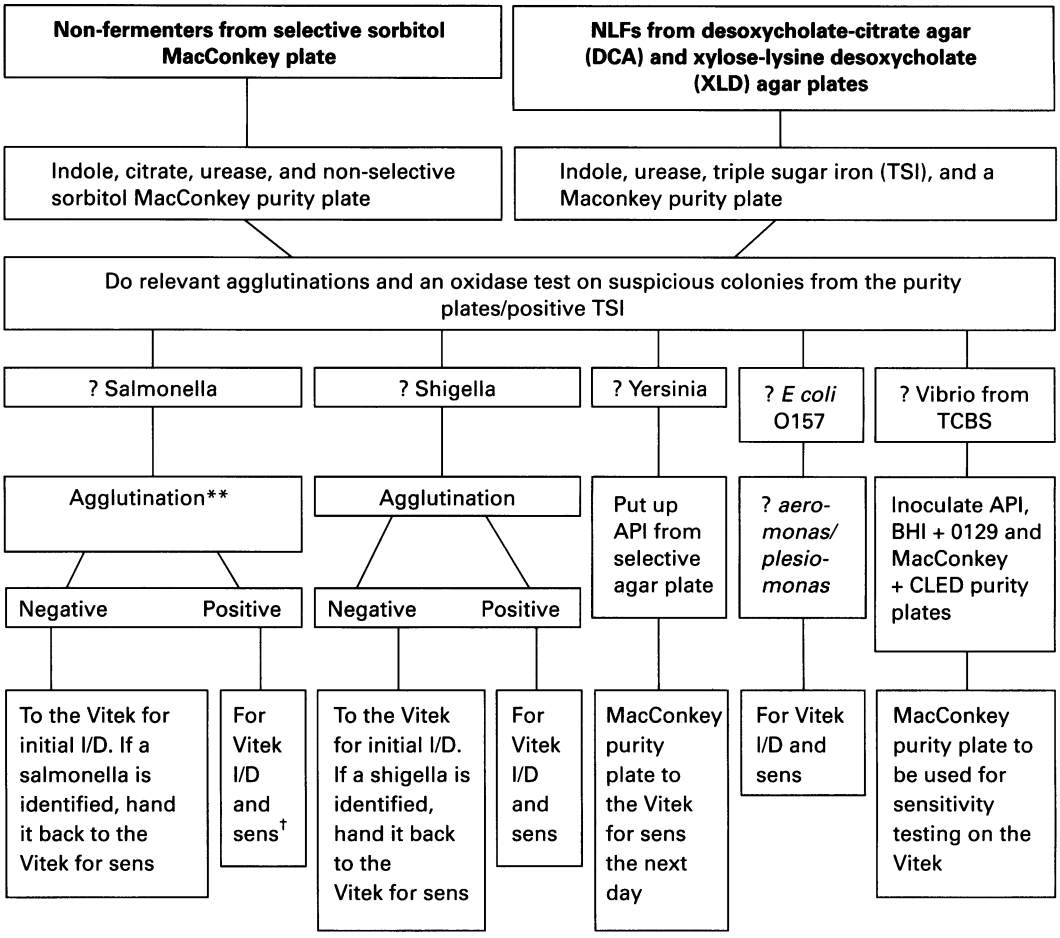
It was initially thought that all Gram negative organisms would be adequately serviced by the GNS PW card (table 2). However, since ordering these custom made cards we were obliged, for control of infection purposes, to change our antibiotic policy on the intensive care unit with regard to Gram negative infections. We withdrew the use of all cephalosporins from this unit and substituted co-amoxiclav and piptazobactam for the cephalosporins. These antibiotics are not available on the GNS-PW card, necessitating the use of manual tests for just these two antibiotics. An important lesson learnt here was that the Vitek cards are not readily amenable to changes in antibiotic policy. It may be prudent therefore to make provision for such changes when ordering custom made cards. Additionally it would have been useful to have an extended spectrum β lactamase (ESBL) testing facility on the GNS-PW card. We have now received a modified Gram negative susceptibility card, the GNS-QL card (table 2), which incorporates ESBL detection, together with the additional antibiotics listed, to include changes in policy as described above.

The Vitek software categorically states that the use of azlocillin is available for pseudomonads only, and will not permit a susceptibility test result on non-pseudomonads to be reported. Sensitivities for antimicrobial agents against *Stenotrophomonas maltophilia* are currently not available, though identification poses no problem.

Since using the GPS-AI and GPS-AH cards (table 2), it has became apparent that the GPS-AH card found little use in the laboratory and as a result of this audit we propose to replace it with the new GPS-AM card (table 2). Additional antimicrobial susceptibility tests now being done manually for MRSA (see II and III in flowchart 5) could justify the need for a second line Gram positive susceptibility card. However, mupirocin—a crucial antibiotic for management of MRSA—is not available in the Vitek system.

*Respiratory, ear, and eye specimens*

As the Vitek analyser does not offer automated identification for *H influenzae* or *M catarrhalis*, the identification and susceptibility testing of




** Should an organism that is indole negative identify as Inactive *E coli* by the Vitek, confirm that it is not *S typhi* by agglutinations before discarding.

† For sensitivities on all faecal pathogens use the new GNS-QL card and report (but suppress) ampicillin, trimethoprim, and ciprofloxacin only.

*Flowchart 6 Identification of faecal pathogens. API, appareil procédes d'identification; BHI, brain-heart infusion; CLED, cysteine-lysine-electrolyte deficient; I/D, identification; NLFs, non-lactose fermenters; TCBS, thiosulphate citrate bile salt.*

these organisms are done by routine methods.[4] Antibiotics such as neomycin, polymyxin, and chloramphenicol are not available on Vitek cards and are done by disc diffusion where appropriate.

*Faeces*

Table 3 lists the antibiotic susceptibility tests done for faecal pathogens by the disc diffusion method. The Vitek does not offer chloramphenicol or nalidixic acid. Because we do not routinely recommend antibiotics for the treatment of non-enteric faecal pathogens, it was agreed that the GNS-PW card would be used, and ampicillin, trimethoprim, and ciprofloxacin be reported only when indicated (flowchart 6).

*MRSA*

Table 3 lists the range of antibiotics currently being used to evaluate MRSA. The GPS-AI card and the more recent GPS-AM card provides first line antistaphylococcal antibiotics. MRSA are tested against the rest by disc diffusion on first isolation and on monthly retesting of known MRSA infected patients. It needs to be emphasised at the outset that the Vitek uses oxacillin and not methicillin, the breakpoints meet with NCCLS standards for sensitive and resistant, and there is no intermediate range. The BSAC does not provide guidelines for the interpretation of staphylococcal susceptibility to oxacillin.

We have met with an interesting finding regarding the identification of MRSA strains (oxacillin resistant) with the Vitek analyser. Over a period of six months we have had strains reported oxacillin resistant by the Vitek (n = 28), and sensitive by methicillin disc testing (n = 19) and the methicillin E-test™ (n = 16). We have therefore opted to retest every new MRSA (oxacillin resistant) strain identified by the Vitek by disc test, using methicillin, and then by E-test™ if necessary, before declaring it to the clinician as an MRSA. All strains showing discrepant results between oxacillin and methicillin susceptibility tests are referred to the Antibiotic Reference Unit for the detection of the mec A gene to confirm true MRSA status. The extra cost and labour is offset by the huge infection control implications and associated expense in wrongly identifying an MRSA in the hospital.

## Expert system

The "Expert system" is a generic term for the genre of computer programs that have the ability to draw inferences and reach certain conclusions, based on carefully formulated information called the knowledge base. The knowledge base in the expert system is composed of data on antimicrobial groupings, organism groupings, and current microbiological information as defined by NCCLS or published scientific data.[1] Adhering strictly to the "knowledge" databases provided by the NCCLS, there are a comprehensive set of rules that help with interpretation of susceptibility test results. The following is an example of an actual rule:

IF
Organism belongs to group 3 "*Enterics*" (citrobacter, enterobacter, klebsiella, serratia, providencia, morganella)
and all aminopenicillins, result = resistant (R)
and any cephalosporins 1, result = R
and all carboxypenicillins, result = S
and all cephalosporins 3, result = S
THEN
Comment: resistance due to probable intrinsic cephalosporinase. Decreased activity may occur with cephalosporins 1 and 2 and some penicillin/inhibitor combinations.
MODIFY FINAL REPORT
*Antibiotic amoxycillin/clavulanic acid, result = moderate (M) or R.
*Antibiotic ampicillin/sulbactam, result = M/R.
*All cephalosporins 1, result = R.
*All cephalosporins 2, result =R.

There are over 60 rules. Reports that fail a rule are flagged up by the system and a microbiologist is then able to study the report in detail and make corrections when necessary.

## The Vitek Data Trac or epidemiology package

The Data Trac is an epidemiology software package that uses data fed into the Vitek to further define organism occurrence, trending, and per cent susceptibility of a range of organisms and antimicrobials. Our experience with the use of this package is limited, largely because the Vitek computer has not been interfaced with the mainframe hospital computer. However, we think this bidirectional link is vital in order to exploit the possibilities of this software package fully.

## Quality control

The Vitek system comes with a stringent quality control program. It involves testing each new batch of identification cards with defined NCTC strains of a range of microorganisms. Quality control tests on the susceptibility cards are done on each batch and with defined strains of known susceptibility.

## Cost implications in using the Vitek system

The Vitek equipment was acquired by the laboratory as a yearly lease, with quarterly payments of a rental fee. In addition a service contract was also purchased by the laboratory. The cost of cards, at present, stands at £3 each for the identification and susceptibility cards. More work is needed in comparing actual Welcan units between conventional testing and Vitek testing in the laboratory.

## Conclusion

The Vitek analyser was found to be a valuable method of automated identification and susceptibility testing of the common microorganisms seen in a busy diagnostic laboratory. Technical and service backup by the company has been prompt and of excellent quality. The undisputed advantage of the system is the speed with which results are available. This can be further augmented if the laboratory computer is interfaced with the hospital mainframe computer. Other benefits are the Expert Rules, the Data Trac epidemiology software, and a stringent if somewhat tedious quality control package. It must be emphasised that the use of the Vitek is optimal when it is used to test the organisms that it identifies with speed and reliability, that is, the common Gram negative organisms (coliforms and pseudomonads) and a selection of Gram positive organisms, as shown on our flow charts. Expecting the system—as we have it in this laboratory—to work as a reference facility to identify uncommon pathogens is placing an unreasonable strain on it.

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